

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AMENDMENT via facsimile
Docket No. GP091-02.UT

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- b) contacting under nucleic acid amplification conditions:
the first single-stranded fusion nucleic acid,
a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and
at least one nucleic acid polymerase activity;
- c) amplifying the fusion nucleic acid in a single isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:
a complementary splice junction site,
a first probe binding site located 3' to and not overlapping the complementary splice junction site, and
a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;
- d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and
- e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

2. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

3. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, wherein the amplifying step includes

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contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a second primer binding site located 3' to both the complementary splice junction and the first probe binding site, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (Reiterated) The method of Claim 1, wherein the oligonucleotide probe binds to the second probe binding site and does not form a stable hybridization complex with the first single-stranded fusion nucleic acid.

5. (Reiterated) The method of Claim 1, wherein the fusion nucleic acid is a *bcr-abl* fusion mRNA and wherein the oligonucleotide probe binds to a *bcr*-derived nucleotide base sequence in the second nucleic acid strands.

6. (Reiterated) The method of Claim 1, wherein step a) includes preparing RNA from the sample containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or chloroform.

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7. (Reiterated) The method of Claim 6, wherein the fusion nucleic acid is mRNA.
8. (Reiterated) The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.
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9. (Amended Four Times) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation consisting essentially of the steps of:
- a) providing a sample containing a fusion mRNA transcript comprising a splice junction;
 - b) contacting under isothermal nucleic acid amplification conditions:
 - the fusion mRNA transcript,
 - a first primer which hybridizes to the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site, and
 - at least one enzyme having nucleic acid polymerase activity;
 - c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:
 - a complementary splice junction site,
 - a first probe binding site located 3' to and not overlapping the complementary splice junction site, wherein the first probe binding site is derived from a second chromosomal region, and
 - a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site is derived from a third chromosomal region and overlaps or is located 3' to sequence complementary to the first primer binding site;
 - d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to

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the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion transcript, thereby forming a hybridization complex of the probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. (Reiterated) The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. (Reiterated) The method of Claim 9, wherein the first probe binding site and the second probe binding site are derived from different locations on the same chromosome in a eukaryotic cell, and the fusion mRNA transcript detected results from an intrachromosomal translocation.

12. (Reiterated) The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript detected results from a translocation involving different chromosomes.

13. (Reiterated) The method of Claim 12, wherein the fusion mRNA transcript results from a translocation of human chromosomes selected from the group consisting of: t(1;19), t(2;5), t(2;13), t(4;11), t(6;9), t(8;21), t(9;11), t(9;22), t(11;14), t(11;19), t(11;22), t(12;21), t(14;18) and t(15;17) translocations.

14. (Reiterated) The method of Claim 13, wherein the fusion mRNA transcript results from a human t(9;22) translocation and the oligonucleotide probe comprises a *bcr*-derived sequence or an *abl*-derived sequence.

15. (Reiterated) One or more oligonucleotides suitable for use in the method of Claim 14, have a

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sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

16. (Reiterated) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. (Reiterated) The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

18. (Reiterated) The method of Claim 9, wherein the amplifying step also amplifies an internal control transcript in the sample by using the first primer and then hybridizing a second oligonucleotide probe which hybridizes to the complement of the internal control transcript but does not hybridize to the complement of the fusion mRNA transcript thereby forming an internal control hybridization complex, and wherein the detecting step also detects the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Reiterated) A method of preparing a sample containing RNA suitable for amplification, consisting essentially of the steps of:

- a) providing a biological sample comprising unpurified RNA;
- b) mixing the biological sample with a solution consisting essentially of:
 - a buffer at a pH of about 6.5 to about 8.5,
 - about 150 mM to about 1M of a soluble salt, and
 - about 0.5% to about 1.5% (v/v) of a non-ionic detergent, to produce a solution containing released RNA;
- c) mixing the solution containing released RNA with a solid support to which is joined an

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immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;

d) separating the hybridization complex joined to the solid support from unhybridized sample components; and

e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. (Reiterated) The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.

21. (New) A method of detecting a fusion mRNA transcript produced as a result of a human *bcr-abl* translocation comprising the steps of:

a) providing a sample containing a human fusion mRNA transcript comprising a *bcr-abl* splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the a second primer which hybridizes to the
complement of the fusion mRNA transcript at a primer binding site derived from a *bcr*
region flanking the *bcr-abl* splice junction site,

a second primer which hybridizes to the complement of the fusion mRNA
transcript at a primer binding site derived from a *bcr* region flanking the *bcr-abl* splice
junction site,

at least one enzyme having an RNA-directed DNA polymerase activity, and

at least one enzyme having an DNA-directed RNA polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses the first primer to produce a second nucleic acid strand complementary to at least a

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portion of the fusion mRNA transcript containing the *bcr-abl* splice junction site,
the second primer to produce a third nucleic acid strand of the same sense as the fusion mRNA transcript containing the *bcr-abl* splice junction site, and
the DNA-dependent RNA polymerase activity to produce amplified RNA that is complementary to the fusion mRNA transcript comprising the *bcr-abl* splice junction;

d) hybridizing the amplified RNA with an oligonucleotide probe which hybridizes to a probe binding site derived from a *bcr* region flanking the *bcr-abl* splice junction, thereby forming a hybridization complex; and

e) detecting the hybridization complex as an indication of the presence of the fusion mRNA transcript in the sample.

22. (New) The method of claim 21, further comprising

in the contacting step, contacting a third primer that hybridizes to the complement of a normal *abl* mRNA transcript at a site located downstream of the first primer in an *abl* sequence present in a normal *abl* mRNA transcript that is replaced by *bcr* sequence in the fusion mRNA transcript,

in the amplifying step, amplifying normal *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript,

in the hybridizing step, hybridizing an oligonucleotide probe that hybridizes to a probe binding site derived from an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, and

in the detecting step, detecting a hybridization complex made up of the probe hybridized to an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, thereby providing an internal control based on amplifying and detecting normal *abl* sequence.

23. (New) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:9 or SEQ ID NO:27 or is a mixture of SEQ ID NO:9 or SEQ ID NO:27.